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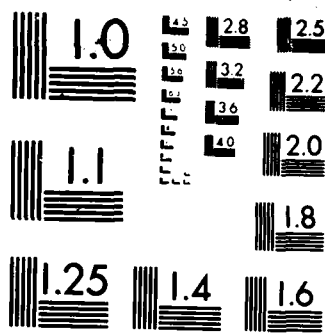
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Form Approved
OMB No 0704-0188
Exp Date Jun 30, 1986

1a REPORT SECURITY CLASSIFICATION Unclassified			1b RESTRICTIVE MARKINGS		
2a SECURITY CLASSIFICATION AUTHORITY			3. DISTRIBUTION / AVAILABILITY OF REPORT Approved for public release; distribution unlimited		
2b DECLASSIFICATION / DOWNGRADING SCHEDULE			5 MONITORING ORGANIZATION REPORT NUMBER(S)		
4 PERFORMING ORGANIZATION REPORT NUMBER(S)			7a NAME OF MONITORING ORGANIZATION		
6a NAME OF PERFORMING ORGANIZATION University of Iowa		6b OFFICE SYMBOL (if applicable)	7b ADDRESS (City, State, and ZIP Code)		
6c ADDRESS (City, State, and ZIP Code) Iowa City, Iowa 52242		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER DAMD17-86-C-6001			
8a NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command		8b OFFICE SYMBOL (if applicable)	10. SOURCE OF FUNDING NUMBERS		
8c ADDRESS (City, State, and ZIP Code) Fort Detrick, Frederick, MD 21701-5012		PROGRAM ELEMENT NO. 62770A	PROJECT NO 3M162. 770A871	TASK NO AH	WORK UNIT ACCESSION NO 366
11 TITLE (Include Security Classification) (U) Rare 2-Substituted Purine Nucleosides					
12 PERSONAL AUTHOR(S) Vasu Nair					
13a TYPE OF REPORT Annual Report		13b TIME COVERED FROM 10/18/85 TO 10/17/86		14. DATE OF REPORT (Year, Month, Day) 1986 October	
15. PAGE COUNT 15					
16. SUPPLEMENTARY NOTATION					
17 COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP			
06	01				
07	03				
19 ABSTRACT (Continue on reverse if necessary and identify by block number)					
<p>This project is concerned with the synthesis of rare 2-substituted purine nucleosides with therapeutic potential against RNA viruses. In the first year of this contract, the goals were to develop rational procedures for the synthesis of the target molecules discussed in the proposal. Synthetic procedures to eight of the target molecules were developed. Two of these compounds, both inosine analogues, were submitted for antiviral evaluation. Four others were in various stages of preparation for submission. In the course of this research investigation conceptually new synthetic methodologies in purine nucleoside chemistry were also discovered.</p>					
20 DISTRIBUTION / AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input checked="" type="checkbox"/> SAME AS RPT <input type="checkbox"/> DTIC USERS					
22a NAME OF RESPONSIBLE INDIVIDUAL Mrs. Judy Pawlus			21 ABSTRACT SECURITY CLASSIFICATION		
			22b TELEPHONE (Include Area Code) 301-663-7325		22c OFFICE SYMBOL SGRD-RMI-S

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Rare 2-Substituted Purine Nucleosides

Annual Report

October 18, 1985 to October 17, 1986

October 1986

Vasu Nair

Supported by

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-86-C-6001

University of Iowa
Iowa City, Iowa 52242

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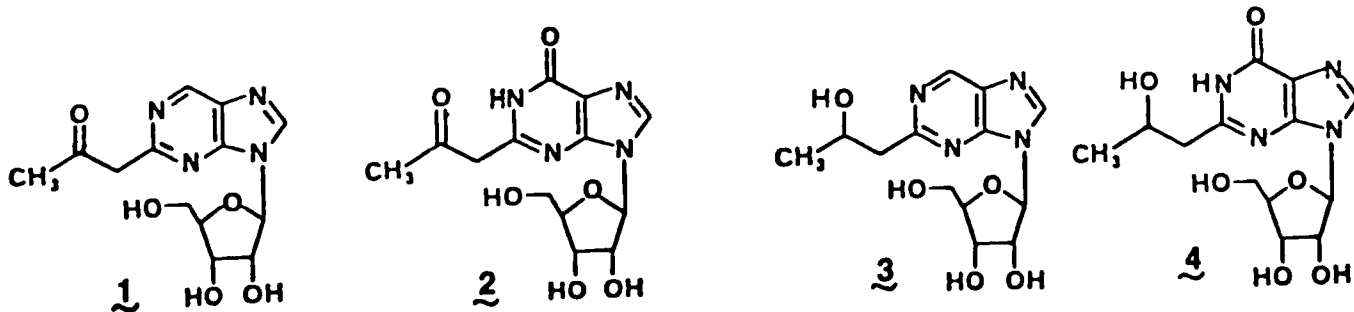


1. Project Title: "Rare 2-Substituted Purine Nucleosides"
2. Name of Contractor: University of Iowa, Iowa City, Iowa 52242
3. Name of Principal Investigator: Vasu Nair
Professor of Chemistry
Phone: (319) 335 1364
4. Reporting Period: October 18, 1985 to October 17, 1986
5. Description of Work Accomplished

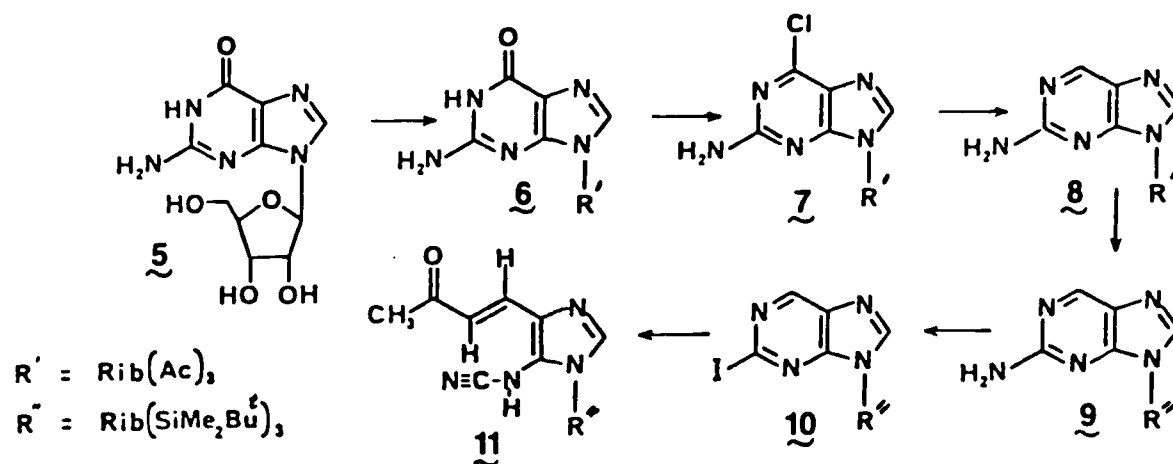
In the first year of this contract, our goals were to develop rational procedures for the synthesis of the target molecules discussed in the proposal. Although difficulties were encountered in approaches studied in the first six months of the contract, these problems were overcome and an excellent and novel methodology for the key step in the synthesis of many of the target compounds was discovered. This approach has been applied successfully to the synthesis of six target compounds, two of which have been submitted with complete data for antiviral evaluation. A description of the synthetic work done during the first year follows.

The starting point of our work involved the preparation of multigram quantities of some basic precursors and investigation of feasible approaches to the synthesis of 2-acetylnebularine 1 and 2-acetylinosine 2. Target molecules 1 and 2 (in their protected form) are direct precursors for the synthesis of 3 and 4, respectively.

The synthesis of compound 1 commenced with guanosine (5) which was converted almost quantitatively (93%) in the first step to 2',3,5'-tri-O-acetylguanosine (6) by treatment with acetic anhydride, dimethylamino-pyridine, and triethylamine in acetonitrile as solvent. The protected nucleoside 6, when treated with phosphorus oxychloride and N,N-diethyl-aniline, was converted to the 2-amino-6-chloropurine nucleoside 7 in about 89% yield. Photolysis of 7 in dry, nitrogen-purged tetrahydrofuran (THF) containing 10% triethylamine produced the 2-amino nucleoside 8 in about 80% yield. This photoinduced reductive dehalogenation has not been reported previously in purine nucleoside chemistry and represents an excellent procedure for the synthesis of 2-aminopurines. Nucleoside 8 is expected to be a key precursor in the synthesis of 1 and 3.



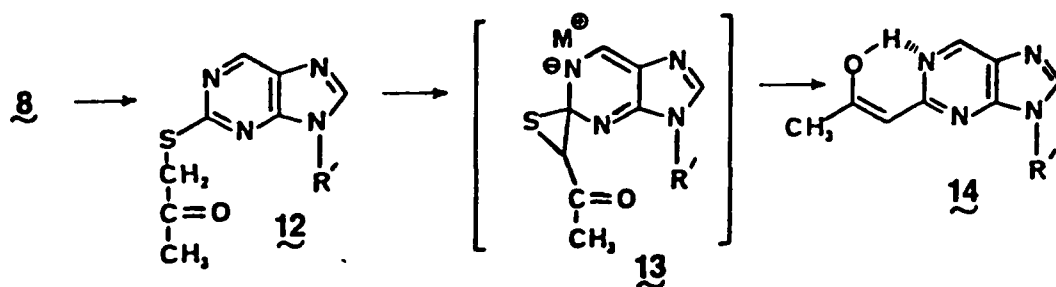
Several approaches were examined for the synthesis of **1** from **8**. The first involved conversion of **8** to its 2-iodinated derivative with subsequent photoinduced $S_{RN}1$ reaction of this iodo compound with the potassium enolate of acetone (Scheme 1). Nucleoside **9** (i.e. the silylated derivative of **8**) was converted to the new 2-iodo-9-(2,3,5-tri-O-t-butyl-dimethylsilyl- β -ribofuranosyl)purine **10** in 67% yield by a deamination-halogenation reaction using *n*-pentyl nitrite, diiodomethane, and trimethylsilyl iodide in hexane. However, when **10** was photolyzed in the presence of the potassium enolate of acetone in THF at -48°C for 20 minutes, the expected $S_{RN}1$ product was not isolated. Careful analysis of the high-field



Scheme 1

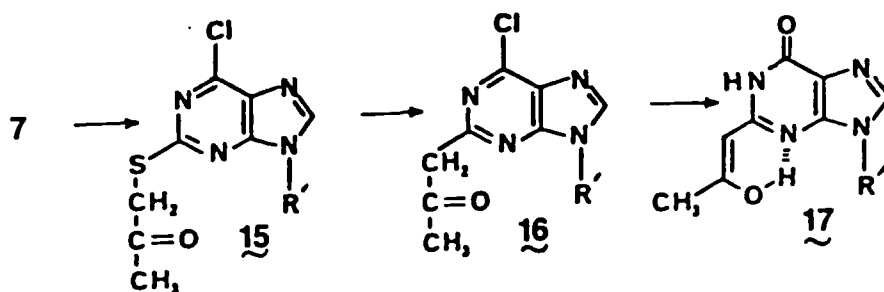
NMR, FTIR, UV, and mass spectral data suggested that nucleophilic attack with subsequent ring opening had occurred at the 6-position to produce **11**. Although this was totally unexpected, failure of the $S_{RN}1$ reaction may be attributed to a marked change in the reduction potential of **10** compared to the corresponding 6-iodo compound. The latter undergoes the $S_{RN}1$ reaction in very good yields. When the 6-position was blocked, as in the case of 2-iodo-6-methoxypurine nucleoside (a precursor for target molecule **2**), the reaction still failed.

An alternative approach to compound **1** also involved the use of the 2-amino nucleoside **8** as a precursor. The methodology involved conversion of **8** to its 2-thioacetyl derivative and subsequent application of the Eschenmoser sulfide contraction on this thio derivative (Scheme 2). Nucleoside **8** can be converted to its 2-thioacetyl derivative **12** in good yields by heating with *n*-pentyl nitrite and diacetyl disulfide in acetonitrile. The Eschenmoser sulfide contraction reaction on **12** to give **14** via the intermediacy of **13** did not proceed as planned under a variety of conditions. Modifications in the experimental procedure included changing the solvent, the base, and the phosphine used.



Scheme 2

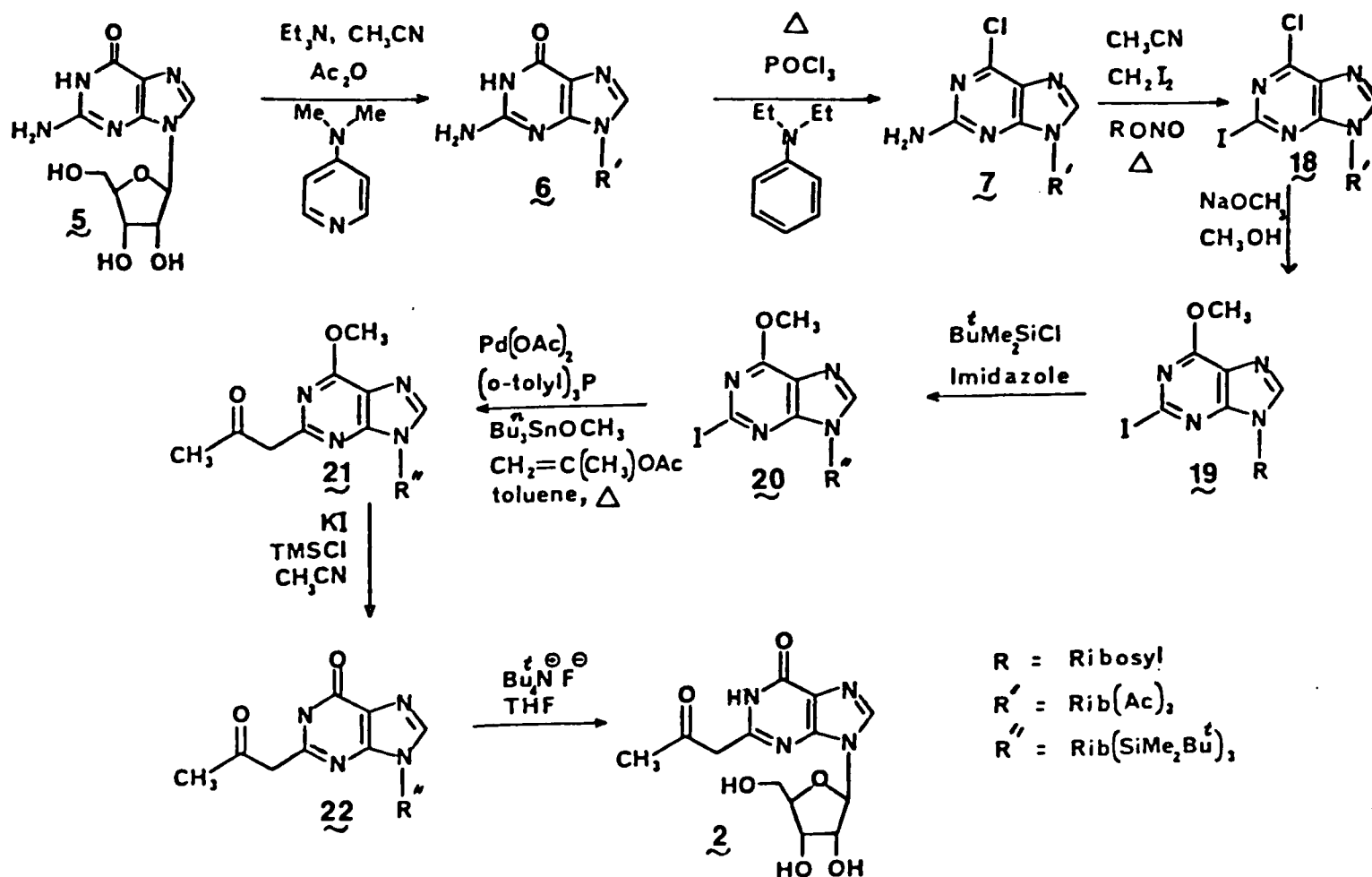
A methodology involving radical addition of thioacetone at the 2-position of an appropriate precursor followed by thermal sulfide contraction and subsequent modification at the 6-position appeared also to be a promising and direct approach to the synthesis of **2** (Scheme 3). However, the sulfide contraction was also unsuccessful in this case.



Scheme 3

Application of the Meerwein reaction and radical coupling reactions did not provide suitable routes to **1** and **2**.

Palladium is known to be able to insert into the carbon-iodine bond of an iodoaromatic system and this intermediate can subsequently undergo cross-coupling reactions with alkenes under suitable conditions. We have been very successful in developing this methodological approach for the synthesis of a number of the target nucleosides for this contract. The methodology is illustrated with the synthesis of **2** (Scheme 4). The synthesis commenced with guanosine (**5**) which was converted almost quantitatively (93%) in the first step to **6** by treatment with acetic anhydride, dimethylaminopyridine, and triethylamine in acetonitrile as solvent. The protected nucleoside **6**, when treated with phosphorous oxychloride and N,N-diethylaniline under carefully controlled conditions, was converted to the 2-amino-6-chloropurine nucleoside **7** in about 89% yield as previously described in this report. This is a considerable improvement for this conversion which is an important reaction in nucleoside chemistry. Reaction of compound **7** with n-pentyl nitrite and diiodomethane in refluxing acetonitrile gave the 6-chloro-2-iodopurine nucleoside **18** in 71% yield. Replacement of the chlorine group at the 6-



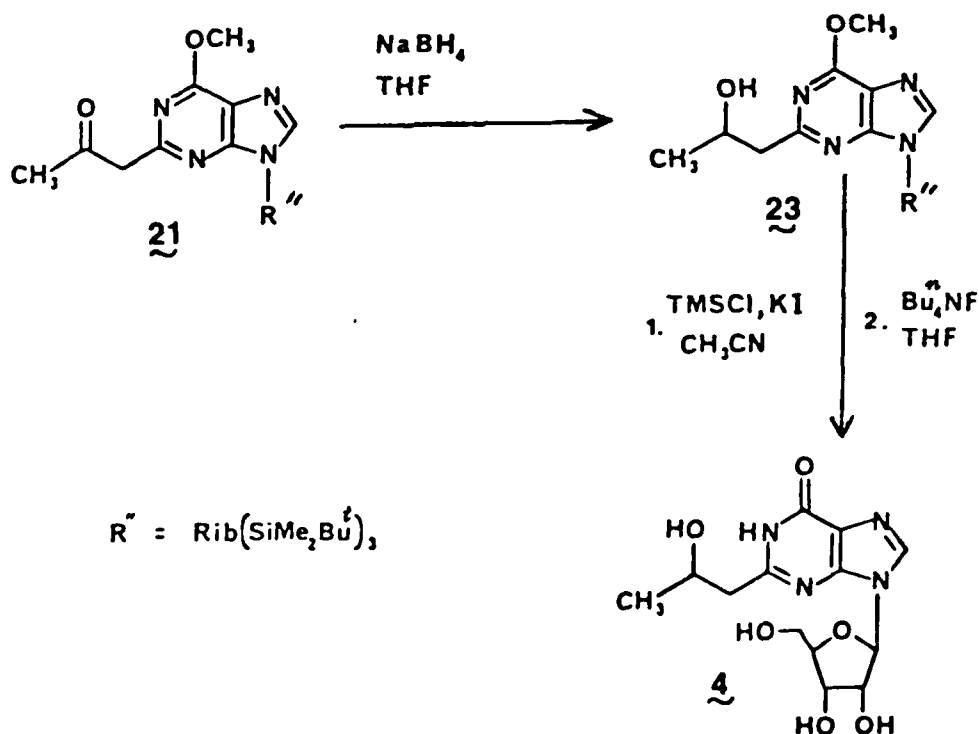
Scheme 4

position with methoxide is accompanied by the desired deprotection of the acetate groups to give 19 in 76% yield. Subsequent protection of the carbohydrate moiety with *t*-butyldimethylsilyl chloride and imidazole in dimethylformamide gave 20 in 96% yield. The key step in the synthesis of 1 was the conversion of 20 to 21 in 70% yield by the palladium catalyzed coupling reaction shown in Scheme 4. This conversion involves insertion of palladium into the carbon-iodine bond of the iodopurine followed by coupling of the derived Pd(II) complex with the tin enolate of acetone, trans-cis isomerization, and reductive elimination to give the product. It is the first example of the use of an Sn reagent in palladium catalyzed

coupling involving nucleosides. The acetonlated nucleoside **21** was converted to **2** in two steps by reaction first with trimethylsilyl iodide (64% yield of **22**) and subsequently with tetrabutylammonium fluoride (93% yield). The overall yield of **2** starting from guanosine was an excellent 17.9%.

Compound **2** was purified to a high degree by reversed-phase high performance liquid chromatography (3 passes) on Amberlite XAD-4 resin using water-ethanol as the eluting solvent. The product may be crystallized from a water-isopropanol mixture. Complete characterization was performed by mass spectrometry, UV, FTIR, and high-field ^1H and ^{13}C NMR spectroscopy. Critical spectral data were presented with the sample submitted for antiviral testing. The high-field ^{13}C NMR spectrum is shown in Fig. 1.

The starting material for the synthesis of compound **4** was the acetonlated nucleoside **21** (Scheme 5). It was smoothly reduced with sodium borohydride in tetrahydrofuran to give the diastereoisomeric products **23** in 75% purified yield. Compound **23** was deprotected to **1** in two steps by reaction first with trimethylsilyl iodide and subsequently with tetrabutylammonium fluoride. The overall yield of **1** starting from guanosine was an excellent 13%. Compound **1** was purified by multiple reversed phase HPLC on Amberlite XAD-4 with 5% ethanol-water as the eluting solvent. Crystallization may be carried out from isopropanol. Complete characterization was carried out by mass spectrometry, UV, FTIR, and high-field ^1H and ^{13}C NMR spectroscopy. The high-field ^{13}C NMR spectrum is enclosed (Fig. 2). Complete spectral data were provided with WRAMC FORM 108.



Scheme 5

Fig. 1 90.6 MHz ^{13}C NMR Spectrum in DMSO-d_6

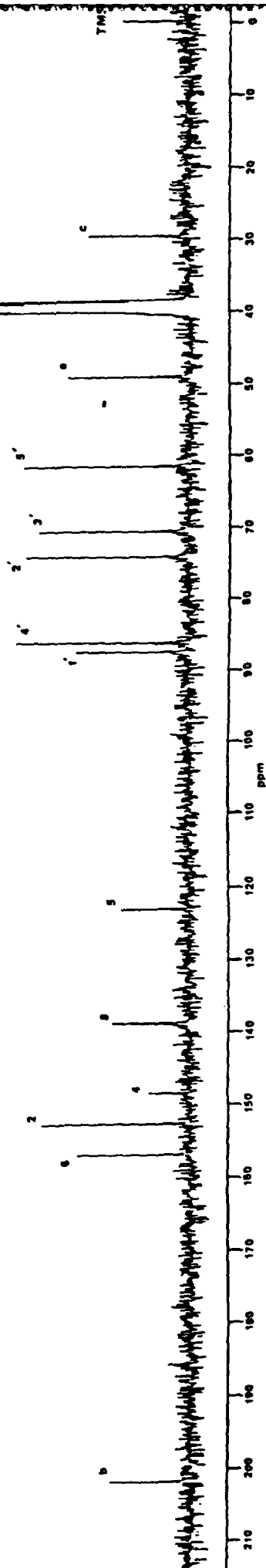
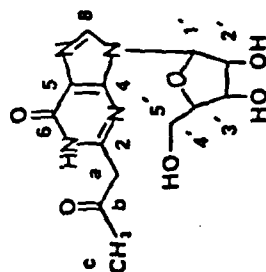
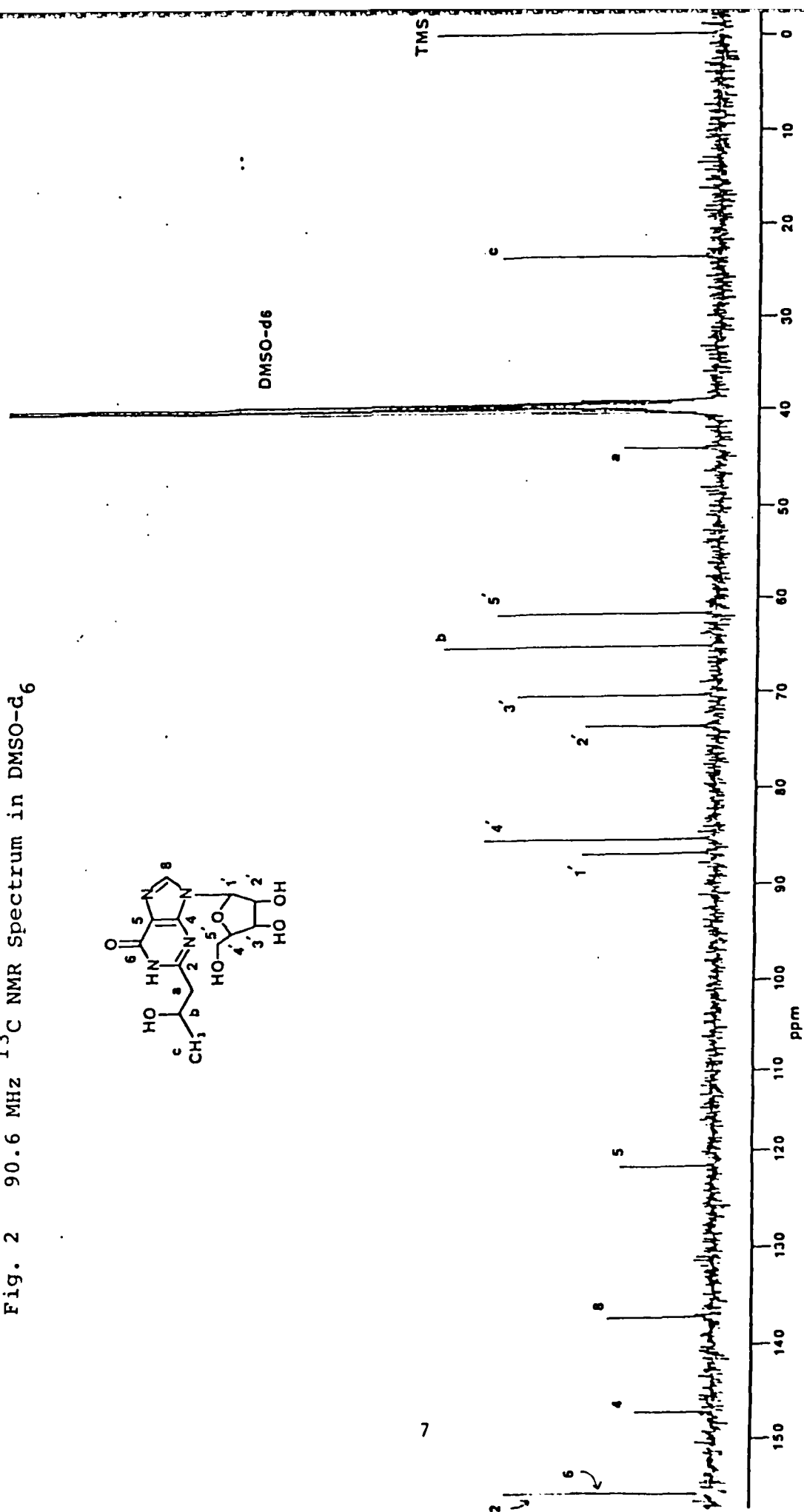
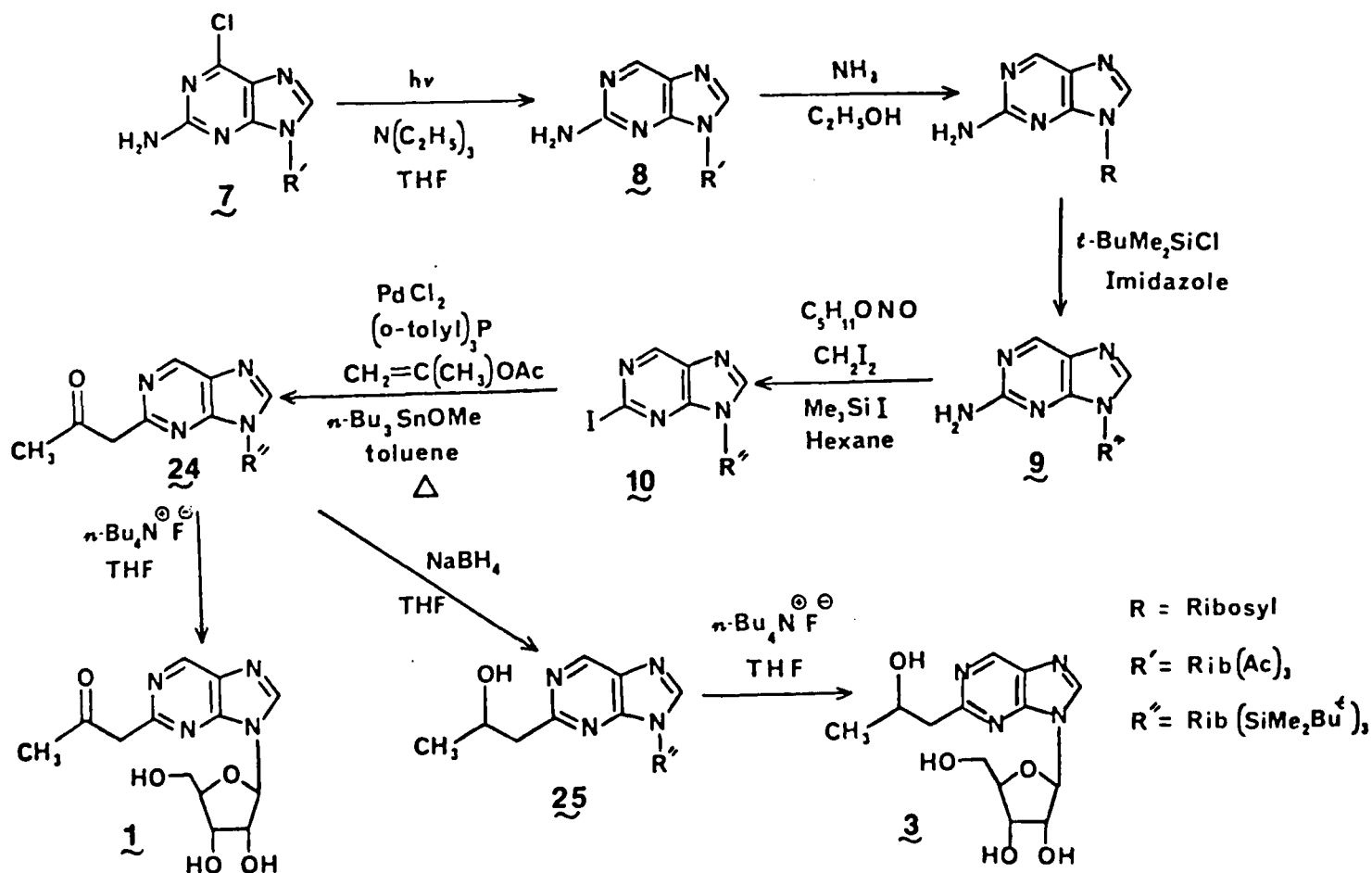


Fig. 2 90.6 MHz ^{13}C NMR Spectrum in $\text{DMSO}-d_6$

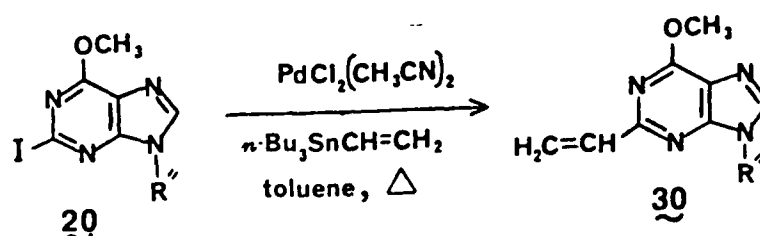
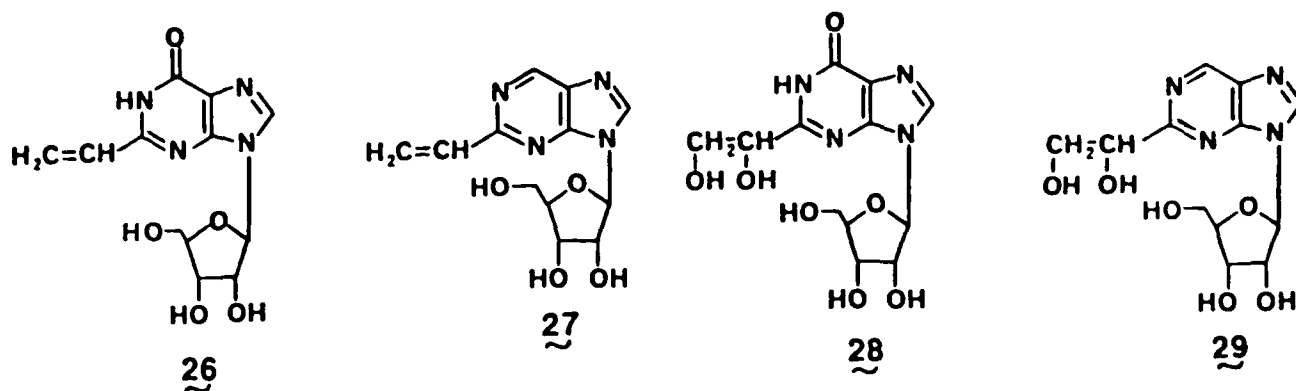


The preparations of 2-acetyl-9-(β -D-ribofuranosyl)purine 1 and 2-(2-hydroxypropyl)-9-(β -D-ribofuranosyl)purine 3 on gram scale are currently being carried out. The methodology developed for these compounds is outlined in Scheme 6. The precursor material for the syntheses was 10, prepared from 7 as described previously (Scheme 1). The palladium catalyzed reaction of 10 with the tributyltin enolate of acetone gave 24 in good yields. Compound 24 can be easily deprotected to 1 with tetrabutylammonium fluoride. It can be reduced with sodium borohydride to 25 which can be deprotected to 3. Both compounds 1 and 3 have been prepared and fully characterized and are now being produced in larger quantities for antiviral evaluation.

Application of our newly developed methodology of palladium catalyzed functionalized alkylation at the 2-position of the purine ring is currently being investigated for the synthesis of target molecules 26, 27, 28, 29. A key reaction is the coupling of vinyl tributyltin with 20 and 10 as shown in Schemes 7 and 8. This reaction proceeds in high yields (87%) in the case of 20 and product 30 has been prepared in gram quantities. Its deprotection has been achieved and target molecule 26 is currently being purified and will be submitted for antiviral evaluation in about two weeks. The hydroxylation of 30 followed by deprotection would give 28 and this conversion is now being examined.

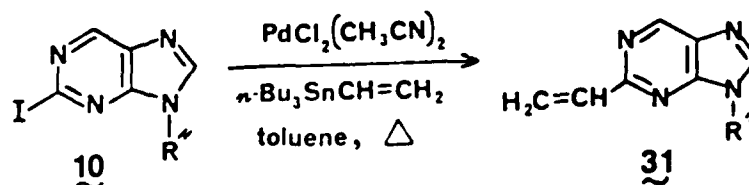


Scheme 6



Scheme 7

Procedures related to the above are also being studied for the synthesis of target molecules 27 and 29 . The conversion shown in Scheme 8 has been completed successfully and is now being scaled up to gram quantities.

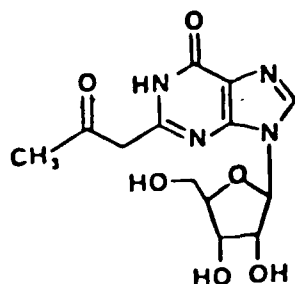


Scheme 8

6. Compounds Submitted and Syntheses Completed:

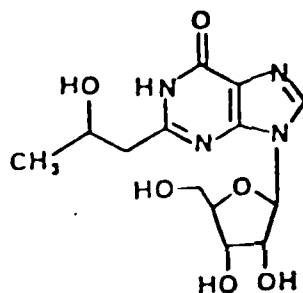
The following compounds were submitted in 1.5 gram quantities to the Department of Antiviral Studies, USAMRIID, Fort Detrick:

- (i) 2-Acetylinosine
or 2-Acetyl-9-(β -D-ribofuranosyl)hypoxanthine



AVS Identifying Number: AVS-002159
Contractor's Identifying Code No: VN-I-101
Report Reference: This Annual Report, Pages 4-5, Scheme 4.

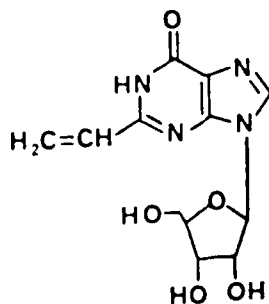
- (ii). 2-(2-Hydroxypropyl)inosine
or 2-(2-Hydroxypropyl)-9-(β -D-ribofuranosyl)hypoxanthine



AVS Identifying Number: AVS-002352
Contractor's Identifying Code No: VN-I-102
Report Reference: This Annual Report, Page 5, Scheme 5.

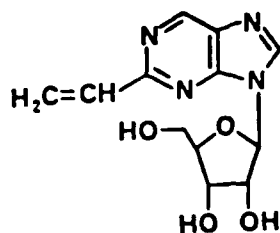
The syntheses of the following compounds have been developed and their gram scale productions are currently being carried out in preparation for submission for antiviral evaluation:

- (iii). 2-Vinylinosine or 2-Vinyl-9-(β -D-ribofuranosyl)hypoxanthine



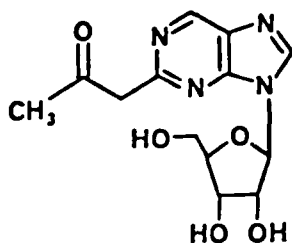
Report Reference: This Annual Report, Page 9, Scheme 7.

(iv). 2-Vinylnebularine or 2-Vinyl-9-(β -D-ribofuranosyl)purine



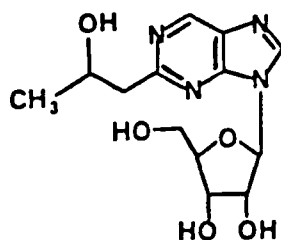
Report Reference: This Annual Report, Page 9, Scheme 8.

(v). 2-Acetylnebularine or 2-Acetyl-9-(β -D-ribofuranosyl)purine



Report Reference: This Annual Report, Page 8, Scheme 6.

(vi). 2-(2-Hydroxypropyl)nebularine or 2-(2-Hydroxypropyl)-9-(β -D-ribofuranosyl)purine



Report Reference: This Annual Report, Pages 8, Scheme 6.

7. Bibliography of Publications and Presentations:

- (i) V. Nair, D. A. Young, and R. DeSilvia, Jr., 2-Halogenated Purine Nucleosides: Synthesis and Reactivity, Submitted to the Journal of Organic Chemistry, 1986 (4 copies furnished to SGRD-RMS).
- (ii) V. Nair, D. A. Young, and R. DeSilvia, Jr., 2-Amino-9-(β -D-ribofuranosyl)purine. Photoinduced Reductive Dehalogenation: A General Approach to 2-Aminopurine and Related Systems, An Invited Article Submitted to "Nucleic Acid Chemistry", Part 4, Edited by L. B. Townsend and R. S. Tipson, 1986 (4 copies furnished to SGRD-RMS).
- (iii) V. Nair, S. D. Chamberlain, R. DeSilvia, Jr., and G. S. Buenger, Synthetic Approaches to Rare 2-Substituted Purine Nucleosides, Submitted to Nucleosides and Nucleotides, 1986 (4 copies furnished to SGRD-RMS).
- (iv) V. Nair, Synthetic Approaches to Rare 2-Substituted Purine Nucleosides, A Lecture at the 7th International Symposium on Nucleosides, Nucleotides, and their Biological Applications, 1986.

8. Personnel Supported:

Stanley D. Chamberlain, Ph.D. Degree, December 1986.
Raymond DeSilvia, Jr., M.S. Degree, August 1986.
Gregory A. Turner
Greg S. Buenger

9. Summary:

Synthetic approaches to eight of the target molecules described in the contract have been developed. Two of these target compounds have already been submitted for antiviral evaluation and four others are currently being prepared in gram quantities for screening studies. In the course of this research investigation we have also discovered conceptually new synthetic approaches to some nucleoside intermediates and final products. This work will have an important impact in synthetic nucleoside chemistry. Three publications have arisen so far from our efforts on this project and a patent application is being prepared. One graduate student on this project will be awarded the Ph.D. degree in December, 1986. Our progress on the contract is right on schedule.

10. Signature and Date:

V. Nair

11-14-86.

END

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